(ii) isolating from the library polynucleotide sequences flanking one side of the inserted transposons to give a first pool of sequences and polynucleotide sequences flanking the other side of the inserted transposons to give a separate second pool of sequences;

(iii) hybridising the first pool of sequences with a first sample of a polynucleotide library from the said organism and the second pool of sequences with a second sample of the said polynucleotide library from the said organism; and

(iv) identifying a polynucleotide in the said polynucleotide library to which at least one of the said pools of polynucleotide sequences does not hybridise, thereby to determine an essential gene of the organism.

30. (New) A method according to claim 29, wherein step (iv) comprises identifying a polynucleotide in the said polynucleotide library to which the said pools of polynucleotide sequences do not hybridise, thereby to determine an essential gene of the organism.

31. (New) A method according to claim 29, wherein the said polynucleotide library is in the form of a gridded array.

32. (New) A method according to claim 29, wherein the organism is a bacterium, yeast, fungus, plant or animal.

33. (New) A method according claim 29, wherein in step (ii) each pool of sequences is isolated by a method comprising:

(a) digesting genomic DNA isolated from a library of transposon-tagged mutants with a restriction endonuclease that cuts within the transposon (T-specific endonuclease) and a second different restriction endonuclease (G-specific endonuclease) which cuts within the disrupted sequence;

(b) ligating the resulting DNA fragments with a linker; and

(c) carrying out PCR on the resulting DNA fragments with an oligonucleotide specific for a transposon sequence and an oligonucleotide specific for a linker sequence.

34. (New) A method according to claim 29, wherein the library of transposon mutants is a library of Tn*phoA* *E. coli* mutants.

35. (New) A method according to claim 34, wherein:

in the isolation of the first pool of sequences the restriction enzyme which cuts in the transposon is *Dra*I and the second enzyme is a 4 base pair restriction endonuclease; and

in the isolation of the second pool of sequences the restriction enzyme which cuts in the transposon is *Hpa*I and the second enzyme is a 4 base pair restriction endonuclease.

36. (New) A method for identifying a conditional essential gene of an organism comprising:

(i) providing a first sample of a library of transposon mutants of the said organism (input library);

(ii) providing a second sample of the library and subjecting that sample to a conditional restraint;

(iii) collecting the mutants that survive the conditional restraint in step (ii) to give a new library (output library); and

(iv) carrying out a method according to claim 29 on the input library from step (i) and on the output library from step (iii), thereby to determine a conditional essential gene of the organism.

37. (New) A method according to claim 36, wherein the organism is a bacterium and the conditional restraint is growth of that bacterium in its host.

38. (New) A method for identifying an inhibitor of transcription and/or translation of an essential gene or a conditional essential gene of an organism and/or an inhibitor of activity of a polypeptide encoded by a said gene, which method comprises:

(a) identifying an essential gene by a method according to claim 29 or a conditional essential gene by a method according to claim 36; and

(b) determining whether a test substance can inhibit transcription and/or translation of a gene identified in (a) and/or activity of a polypeptide encoded by a said identified gene, thereby to identify a said inhibitor.

39. (New) An inhibitor identified by a method according to claim 38.

40. (New) An inhibitor according to claim 39, wherein the essential or conditional essential gene in claim 38 is a bacterial, fungal or eukaryotic parasite essential or conditional essential gene.

41. (New) A pharmaceutical composition comprising an inhibitor according to claim 40 and a pharmaceutically acceptable carrier or diluent.

42. (New) A method for the preparation of a pharmaceutical composition, which method comprises:

(a) identifying an inhibitor of transcription and/or translation of an essential gene or conditional essential gene of an organism and/or an inhibitor of activity of a polypeptide encoded by a said gene, by a method according to claim 38, wherein the essential or conditional essential gene is a bacterial, fungal or eukaryotic parasite essential or conditional essential gene; and

(b) formulating an inhibitor identified in step (a) with a pharmaceutically acceptable carrier or diluent.

43. (New) A method of treating a host suffering from a bacterial, fungal or eukaryotic parasite infection, which comprises administering to the host a therapeutically effective amount of an inhibitor according to claim 40.

44. (New) An inhibitor according to claim 39, wherein the essential or conditional essential gene in

claim 38 is a plant bacterial, plant fungal or plant pest essential or conditional essential gene.

45. (New) A plant bactericide, plant fungicide or plant pesticide which comprises an inhibitor according to claim 44 and an agriculturally acceptable carrier or diluent.

46. (New) An inhibitor according to claim 39, wherein the essential or conditional essential gene in claim 38 is a plant essential or conditional essential gene.

47. (New) A herbicide which comprises an inhibitor according to claim 46 and an agriculturally acceptable carrier or diluent.

48. (New) A bacterium attenuated by a non-reverting mutation in one or more genes identified by a method as defined in claim 37.

49. (New) A method for the preparation of an attenuated bacterium, which method comprises:

(a) identifying a conditional essential gene in a bacterium by a method according to claim 37; and

(b) introducing a non-reverting mutation into a conditional essential gene identified in (a) of the bacterium, thereby to attenuate the bacterium.

50. (New) A vaccine comprising a bacterium according to claim 48 and a pharmaceutically acceptable carrier or diluent.

51. (New) A method for the preparation of a vaccine, which method comprises:

(a) identifying a conditional essential gene in a bacterium by a method according to claim 37;

(b) introducing a non-reverting mutation into a conditional essential gene identified in (a) of the bacterium, thereby to attenuate the bacterium; and

(c) formulating the attenuated bacterium prepared in (b) with a pharmaceutically acceptable carrier or diluent.

52. (New) A method of raising an immune response in a mammalian host, which comprises administering to the host a bacterium according to claim 48 or a vaccine according to claim 50.

53. (New) A method for raising an immune response in a host, which method comprises:

(a) identifying a conditional essential gene in a bacterium by a method according to claim 37;

(b) introducing a non-reverting mutation into a conditional essential gene identified in (a) of the bacterium, thereby to attenuate the bacterium;

(c) formulating the attenuated bacterium prepared in (b) with a pharmaceutically acceptable carrier or diluent; and

(d) administering to the host the attenuated bacterium formulated in (c).

REMARKS

Favorable consideration of this application and entry of the foregoing amendments are respectfully requested.

In considering the newly presented claims, it will be appreciated that claims 1 and 4 have been combined and the phrase "at least one of the said pools of" has been added to part (iv) to give new claim 29. The basis for the introduction of the phrase "at least one of the said pools of" can be found at page 14, lines 17 to 25 and Figure 2 of the application as filed. The passage at page 14 and